transplant, disease status, donor type, absolute neutrophil count, absolute lymphocyte count (ALC), presence of circulating blasts, stem cell source, CD34+ cell dose, conditioning intensity, cytogenetic profile, antecedent myelodysplastic syndrome (MDS) or secondary AML, and development of acute graft versus host disease (GVHD). Deaths attributable to relapse versus non-relapse causes were recorded for each PFT group.

Results: Pre-transplant characteristics included a median age of 49 yrs (range 7–69 yrs), intermediate/high risk cytogenetics (95%), CR1/ CR2 (93%), circulating blasts (8%), full intensity conditioning (78%), related donor (48%), FEV1< 80% (12%), FVC < 80% (15%), and DLCO < 65% (34%). Diminished FEV1 (< 80%), disease status, regimen intensity, ALC, and circulating blasts were associated with a significantly reduced disease free survival (Table). Though FEV1 < 80% and FVC < 80% were both associated with a poor DFS (p = 0.001 and p = 0.027) and OS (p = 0.005 and p = 0.017) by univariate analysis, only FEV1 < 80% remained associated with DFS and OS in the multivariate Cox regression analysis. Pre-transplant DLCO < 65% did not reach significance for either DFS or OS by univariate or multivariate analysis. Relapse related mortality was more common in patients with a pre-transplant FEV1 ≥80% (50%) then patients with an FEV1 <80% (39%).

Conclusion: Pre-transplant FEV1 < 80% is associated with reduced DFS and OS in patients with AML undergoing allogeneic HSCT and may provide valuable prognostic information in pre-transplant evaluations.

Pre-Transplant Factors Associated with Survival

	Overall Survival		Disease Free Survival	
Risk Factor	Risk Ratio	p-value	Risk Ratio	p-value
FEV1≥ 80	0.519	0.019	0.467	0.006
Disease Status[1]	2.144	0.000	1.924	0.004
Regimen Intensity [2]	2.174	0.001	2.047	0.003
ALC ≥ 0.5	0.513	0.003	0.544	0.008
Peripheral Blast +[3]	-	-	2.220	0.018

[1]Disease >CR2 or in relapse.
[2]reduced intensity conditioning (RIC).
[3]not associated with overall survival.

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ALLOGENEIC STEM CELL TRANSPLANTION IN RELAPSED/REFRACTORY ACUTE LEUKEMIA: A LONG TERM SINGLE INSTITUTION EXPERIENCE

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Introduction: The clinical outcomes of relapsed and refractory acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) are dismal in adults treated with conventional therapy. The majority of patients with relapsed ALL and AML who are subsequently cured are transplanted in second remission or, occasionally, beyond second complete remission. A minority of patients, in the range of 10 to 20%, with relapsed/refractory disease are cured by an allogeneic bone marrow transplant.

Objective: We retrospectively examined 137 patients with relapsed and/or refractory acute leukemia who were recipients of allogeneic transplants (matched related [MRD], matched unrelated [MUD] and umbilical cord blood [UCB]) at our center from August 1986 to August 2008 to determine the outcome in advanced leukemia.

Methods: One hundred thirty seven patients (AML, n = 95 and ALL, n = 42) received allogeneic transplants including: 75 MRD, 39 MUD, and 23 UCB. Conditioning regimens were mostly myeloablative using combinations of cyclophosphamide(Cy), busulfan (Bu), etoposide(VP) and total body irradiation (TBI): TBI/Cy/VP, n = 31; TBI/Cy, n = 66; other TBI based, n = 10; Bu/Cy, n = 20;.other, n = 9. GVHD prophylaxis varied over the years but included a calcineurin inhibitor with methotrexate for MUD/MRD transplants and steroids for UCB transplants.

Results: The median age at transplant was 45 years for AML (13–72) and 32 years for ALL (1–66). Results are shown in the table below and compared to the survival data (extrapolated) from the NMDP/ CIBMTR database.

Table 1 Transplant Outcomes in Relapsed and/or Refractory Acute Leukemia

	N	100 day survival	1 year survival	2 year survival	NMDP/ CIBMTR -MRD	NMDP/ CIBMTR -MUD
					1 year–2year	1 year–2year
AML	95	62%	33%	27%		
MRD	75	68%	30%	22%	40%–30%	
MUD	39	54%	30%	26%		30%–20%
UCB	23	48%	26%	16%		
Survival by Age						
<40yrs	35	74%	48%	38%		
40–55yrs	36	56%	29%	26%		
>55yrs	24	54%	12%	12%		
ALL	42	57%	21%	11%	35%–20%	30%–15%

Conclusion: Our single institutional experience in allogeneic transplantation for advanced leukemia appears comparable to the CIBMTR/NMDP database. Younger patients had the best outcomes. MUD recipients had comparable outcomes to MRD. Therefore, we recommend that younger patients with advanced leukemia be strongly considered for allogeneic transplant, regardless of stem cell source, as the only potentially curative option.

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SELECTIVE REPROGRAMMING OF CD19-SPECIFIC T CELLS WITH IL-21 AND CD28 SIGNALING FOR ADOPTIVE IMMUNOTHERAPY OF ACUTE LYMPHOBLASTIC LEUKEMIA

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Adoptive transfer of T cells has been used to treat and prevent malignancies and opportunistic infections. To improve therapeutic efficacy, investigators initially redirected specificity through the introduction of immunoreceptors. Early-phase trials are now underway to demonstrate the safety and feasibility of CD19-specific chimeric antigen receptors (CARs) which recognize antigen independent of MHC. However, it is recognized that improving *in vivo* persistence will be needed to enhance therapeutic potential of CAR^+ T cells. To this end we developed two approaches: (i) *intrinsic:* altering the signalling pathway of T cells to improve persistence and (ii) *extrinsic:* altering the culturing milieu to numerically expand CAR^+ T cells with a proliferative advantage. Previously we reported on a 2nd generation CAR (designated CD19RCD28) which activates T cells through CD3-ζ and CD28 endodomains, to sustain proliferation of CD19-specific T cells. One extrinsic factor to be assessed is IL-21, a member of the common γ-chain receptor cytokine family that can signal $CD8^+$ T cells in conjunction with CD28 to support proliferation and acquisition of desired effector functions. We now report that the addition of exogenous IL-21 favors the microenvironment to selectively propagate CAR^+ $CD8^+$ T cells with ability to kill $CD19^+$ tumor targets. To evaluate this role for IL-21, peripheral blood-derived T cells were electroporated with CD19RCD28 CAR expressed as a *Sleeping Beauty* (SB) transposon and propagated in the presence of IL-2 and/or IL-21 on γ-irradiated $CD19^+$ artificial antigen presenting cells (aAPC). There was a selective outgrowth of CAR^+CD8^+ T cells when IL-21 was present in the culture medium, compared with predominant outgrowth of CAR^+CD4^+ T cells when IL-21 was absent (Table). The propagated CAR^+ T cells produced more IFN-γ in response to $CD19^+$ stimulator cells compared to cells cultured in parallel only on IL-2, and displayed a central memory surface phenotype while retaining an ability to exhibit CD19-dependent cytolysis.

These data demonstrate that a combination of intrinsic and extrinsic approaches can be used to shape a population of CAR^+ T cells that exhibit redirected specificity for CD19. Since the SB system has achieved regulatory approval and the aAPC are being manufactured to clinical grade through PACT/NHLBI, investigators are now able to infuse cytolytic $CD8^+$ and $CD4^+$ T cells expressing markers of central memory and ability to sustain proliferation.

Expression of CAR on $CD8^+$and $CD4^+$ T cells propagated on aAPC with IL-2 +/- IL-21

Culture conditions	$CD4^+CAR^+$	$CD8^+CAR^+$
IL-2	42%	1%
IL-2 + IL-21	22%	76%

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THE INTERACTION BETWEEN LEUKEMIA AND BONE MARROW STROMA PROTECTS THE TUMOR CELLS FROM CHEMOTHERAPY- AND RADIOTHERAPY-INDUCED APOPTOSIS

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Introduction: The tumor microenvironment is increasingly being recognized as a critical factor in mediating cancer development and drug resistance. Despite a high response rate to chemotherapy, the vast majority of patients with AML are destined to relapse due to residual disease in the bone marrow (BM). We have previously reported that AMD3100 (CXCR4 inhibitor) produces a rapid and transient leukemia cell mobilization from BM into peripheral blood, sensitizing leukemia cells to chemotherapy (ASH 2006, Nervi et al).

Objectives: To evaluate the effect of the interaction between APL and BM stroma in the expansion, survival and chemo/radio sensitization of leukemia cells.

Results: We used a mouse leukemia cell line generated by knocking in the human PML-RARα cDNA (APL). To evaluate the physical interaction between APL cells and the bone marrow stromal cells (BMSC), 5×10^4 APL cells were cultured for 24h without or with a BMSC monolayer (M2-10B4), or fibroblast monolayer (L-cell). We observed 0%, 80% and 15% respectively ($p<0.001$). To investigate the chemo/radio-sensitivity of APL cells, the latter were cultured without and with BMSCs for 24h followed by the addition of various increasing concentrations of AraC for 24h or radiotherapy (xRt). Flow cytometric analysis of GR1+ cells using apoptotic indicators such as Bcl-2, Annexin V and caspase-3 revealed a significant protection from cell death of the APL cells by the BM stroma. Furthermore, the analysis of the proliferative state of these APL cells with or without stroma by flow cytometry using BrDU, CFSE and PI showed a decreased cellular proliferation of APL cells in the presence of BM stroma (7% proliferates with stroma versus 70% without stroma). N = 24 mice were injected with 10^6 APL cells iv and after 14 days the mice had an average of 10% APL cells in the peripheral blood. Eight mice received 300cGy xRt in day 16, and 8 mice received the combination of 300cGy + AMD3100 (1h before and 3h later xRt to interrupt APL/stroma interaction). As a control group 8 mice were left untreated. The mean survival for the untreated, xRt, and xRt + AMD were 21, 26 and 29 days respectively ($p<0,02$).

Conclusion: BM microenvironment recruits APL cells to G0 and increases antiapoptotic signals that protects leukemia cells from apoptosis mediated by cytotoxic agents. The interruption of this interaction may improve outcomes in leukemia therapy.

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OUTCOMES OF ALLOGENEIC STEM CELL TRANSPLANTATION FOR PATIENTS WITH ACUTE LEUKEMIA TRANSFORMED FROM MYELOFIBROSIS

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Background and Rationale: Transformation of myelofibrosis (MF) to acute leukemia (>20% blasts) portends a grave prognosis. Allogeneic stem cell transplantation (ASCT) is a curative approach for patients with acute leukemia; however, the outcomes of ASCT in patients with MF transformed to acute leukemia are currently unknown.

Methods: Fifty-one consecutive patients with either primary (PMF) or secondary (SMF) were transplanted at UTMDACC after 1994. Thirteen patients who developed AML (25%), 10 arising from PMF and 3 with SMF, received an ASCT from a sibling or matched unrelated donor. Median age was 59 years. Five patients (38%) had prior splenectomy. JAK2V617F mutation analysis was performed in 7, and was present in 5 patients. Cytogenetics were intermediate in 10 and poor-risk in 3 patients. Seven patients (54%) were not in remission at the time of transplant. Eleven of 13 patients received induction chemotherapy; 6 achieved remission, while 7 had persistent disease at the time of transplant. One patient had a prior autologous transplant and 2 patients had prior allogeneic transplant for myelofibrosis. The donors were matched siblings (7 patients), matched unrelated (4 patients) and 1 antigen mismatched relatives (2 patients). The stem cell source was peripheral blood in 9 and bone marrow in 4 patients. Nine patients received a reduced-intensity conditioning regimen with a fludrabine-melphalan-based regimen, and 4 myeloablative conditioning (3 fludarabine-busulfan, 1 busulfan-cyclophosphamide).

Results: All patients engrafted, 75% achieved full donor chimerism, on day 30. Neutrophil and platelet engraftment occurred after a median of 13 and 21.5 days. Twelve evaluable patients achieved remission; 3 subsequently relapsed. JAK2V617F mutation became negative after transplant in all tested patients and reappeared in 1 patient who later relapsed. Grade 2–4 aGVHD developed in 3 patients (grade 3–4 in one) and cGVHD in 4/11 evaluable patients (extensive in two). After a median follow-up of 17.2 months (range 7.2–128.6 mo), OS and EFS were 49% (SE 15%) and 44% (SE 14%), respectively. Six patients died, related to disease relapse (2), pneumonia (2), GVHD (1), and hepatic failure (1).

Conclusion: Patients with acute leukemia transformed from myelofibrosis with good performance status can achieve durable complete remissions with ASCT.

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TOLERABILITY AND OUTCOMES OF A TARGETED INTRAVENOUS BUSULFAN AND FLUDARABINE (T-BU/FLU) CONDITIONING REGIMEN FOR THE TREATMENT DE NOVO AND SECONDARY ACUTE MYELOID LEUKEMIA (AML)

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In an effort to reduce non-relapse mortality (NRM) and improve survival in patients with AML we evaluated targeting busulfan to daily AUC of 5300mmol/min/day. Since 2004 we have treated 100 patients, with *de novo* (66%) or secondary AML (34%), with a median age of 48 years (range 21–68) with a targeted IV busulfan plus fludarabine (t-Bu/Flu) reduced intensity regimen. 52 patients were in CR1, 23 in CR2 and 25 inadvanced AML. Of those in CR1, 50% had intermediate-risk cytogenetics (IRC) and 50% had unfavorable-risk cytogenetics (URC) at diagnosis. The patients were treated with t-BuFlu (Bu: 130–145 mg/m^2/day on days 1 and 2 with pharmacokinetic targeting for days 3 and 4; Flu 40mg/m^2/day for 4 days). A median Bu AUC of 5265mmol/min/day was achieved. Patients received matched sibling (38%), unrelated (40%), mismatched unrelated (21%) or mismatched related (1%) HCT. GVHD prophylaxis consisted of tacrolimus plus methotrexate or mycophenolate mofetil. Mismatched recipients received rabbit ATG 7.5mg/kg as additional GVHD prophylaxis.

Results: NRM was 5% at 100 days and 17% at 1 year. Relapse rates for patients in CR1 were 38.5 and 42.3 % (p = ns) for the IRC and URC, respectively. With a median follow-up of 1 year, EFS was 54% and OS was 57% at 1 year. There was a lower EFS (63 vs. 30%, p = 0.04) and a trend towards better OS (64 vs. 46%, p = 0.07) for patient transplanted in CR1 compared to those beyond first CR. There was no difference in the outcomes in EFS (p = 0.56) or OS (p = 0.98) between the IRC (n = 56) and URC groups (n = 40). Patients in first or second remission (n = 74) had a better OS (62 vs. 40%, p = 0.04) than those not in remission (n = 26) at the time transplant. We noted a slight difference in outcomes based on age below (n = 66) or above (n = 34) 55 years, but neither reached statistical significance (EFS 58 vs. 44%, p = 0.17; OS 60 vs. 49%,